A new Species of *Mugilicola* Tripathi (Copepoda: Poecilostomatoida) and a Review of the Family Therodamasidae

GEOFFREY A. BOXSHALL

(Communicated by C. N. SMITHERS)

BOXSHALL, G. A. A new species of *Mugilicola* Tripathi (Copepoda: Poecilostomatoida) and a review of the family Therodamasidae. *Proc. Linn. Soc. N.S.W.* 108 (3), (1985) 1986: 183-186.

A new species of *Mugilicola* is described from the gills of *Sillago ciliata* caught off the coast of New South Wales. *Mugilicola* and *Paeonodes* are closely related but neither exhibits the same tagmosis as *Therodamas*, the type genus of the family Therodamasidae to which all three genera have been referred. There is no apparent justification for retaining the Therodamasidae as a separate family since these genera can be regarded as highly transformed representatives of the Ergasilidae.

G. A. Boxshall, Department of Zoology, British Museum (Natural History), Cromwell Road, London SW7 5BD, England; *manuscript received 27 November 1984, accepted for publication 17 April 1985.*

KEY WORDS: parasitic copepod — Therodamasidae — Australia — fish host.

INTRODUCTION

The genus *Mugilicola* was established by Tripathi (1960) to accommodate a copepod parasite of two species of *Mugil* from India. Tripathi placed *Mugilicola* in a new family, the Therodamasidae, based on the genus *Therodamas* Krøyer, 1863. This family is closely related to the Ergasilidae, as recognized by Thomsen (1949) and Tripathi (1960). Cressey (1972), in his discussion of the genus *Therodamas*, suggested that it might be accorded subfamilial separation within the family Ergasilidae. Hewitt (1969) enlarged the Therodamasidae by the transfer of the genus *Paeonodes* Wilson, 1944. *Paeonodes* and *Mugilicola* share the same tagmosis and are closely related but their relationship with the type genus, *Therodamas*, is slight. The discovery of a third species of *Mugilicola* from Australia stimulated this review of the family.

MATERIALS AND METHODS

A single female was collected from the gills of a whiting, *Sillago ciliata* Cuvier & Valenciennes, 1829, caught off Arrawarra Beach, New South Wales, Australia. The specimen was part of an extensive collection made by Klaus Rohde (University of New England, Armidale) from fishes of southeastern Australia. The holotype ♀ is deposited in the collections of the British Museum (Natural History), Reg. No. 1984.189. The specimen was dissected and examined in lactophenol. Drawings were made using an Olympus BH-2 microscope and drawing tube.

Mugilicola australiensis new species

Description: Body of adult female (Fig. 1**A**) highly transformed and lacking any obvious external segmentation. Head small, widest posteriorly but without cephalic lobes. Neck long and slender comprising over 60 per cent of the total body length and merging imperceptibly with the broader trunk. Trunk bearing first legs just posterior to its midlevel, legs 2 and 3 on its posterior surface (Fig. 1**B**). Small urosome (Fig. 1**C**) consisting

A new Species of *Mugilicola* Tripathi (Copepoda: Poecilostomatoida) and a Review of the Family Therodamasidae

GEOFFREY A. BOXSHALL

(Communicated by C. N. SMITHERS)

BOXSHALL, G. A. A new species of *Mugilicola* Tripathi (Copepoda: Poecilostomatoida) and a review of the family Therodamasidae. *Proc. Linn. Soc. N.S.W.* 108 (3), (1985) 1986: 183-186.

A new species of *Mugilicola* is described from the gills of *Sillago ciliata* caught off the coast of New South Wales. *Mugilicola* and *Paeonodes* are closely related but neither exhibits the same tagmosis as *Therodamas*, the type genus of the family Therodamasidae to which all three genera have been referred. There is no apparent justification for retaining the Therodamasidae as a separate family since these genera can be regarded as highly transformed representatives of the Ergasilidae.

G. A. Boxshall, Department of Zoology, British Museum (Natural History), Cromwell Road, London SW7 5BD, England; *manuscript received 27 November 1984, accepted for publication 17 April 1985.*

KEY WORDS: parasitic copepod — Therodamasidae — Australia — fish host.

INTRODUCTION

The genus *Mugilicola* was established by Tripathi (1960) to accommodate a copepod parasite of two species of *Mugil* from India. Tripathi placed *Mugilicola* in a new family, the Therodamasidae, based on the genus *Therodamas* Krøyer, 1863. This family is closely related to the Ergasilidae, as recognized by Thomsen (1949) and Tripathi (1960). Cressey (1972), in his discussion of the genus *Therodamas*, suggested that it might be accorded subfamilial separation within the family Ergasilidae. Hewitt (1969) enlarged the Therodamasidae by the transfer of the genus *Paeonodes* Wilson, 1944. *Paeonodes* and *Mugilicola* share the same tagmosis and are closely related but their relationship with the type genus, *Therodamas*, is slight. The discovery of a third species of *Mugilicola* from Australia stimulated this review of the family.

MATERIALS AND METHODS

A single female was collected from the gills of a whiting, *Sillago ciliata* Cuvier & Valenciennes, 1829, caught off Arrawarra Beach, New South Wales, Australia. The specimen was part of an extensive collection made by Klaus Rohde (University of New England, Armidale) from fishes of southeastern Australia. The holotype ♀ is deposited in the collections of the British Museum (Natural History), Reg. No. 1984.189. The specimen was dissected and examined in lactophenol. Drawings were made using an Olympus BH-2 microscope and drawing tube.

Mugilicola australiensis new species

Description: Body of adult female (Fig. 1**A**) highly transformed and lacking any obvious external segmentation. Head small, widest posteriorly but without cephalic lobes. Neck long and slender comprising over 60 per cent of the total body length and merging imperceptibly with the broader trunk. Trunk bearing first legs just posterior to its midlevel, legs 2 and 3 on its posterior surface (Fig. 1**B**). Small urosome (Fig. 1**C**) consisting

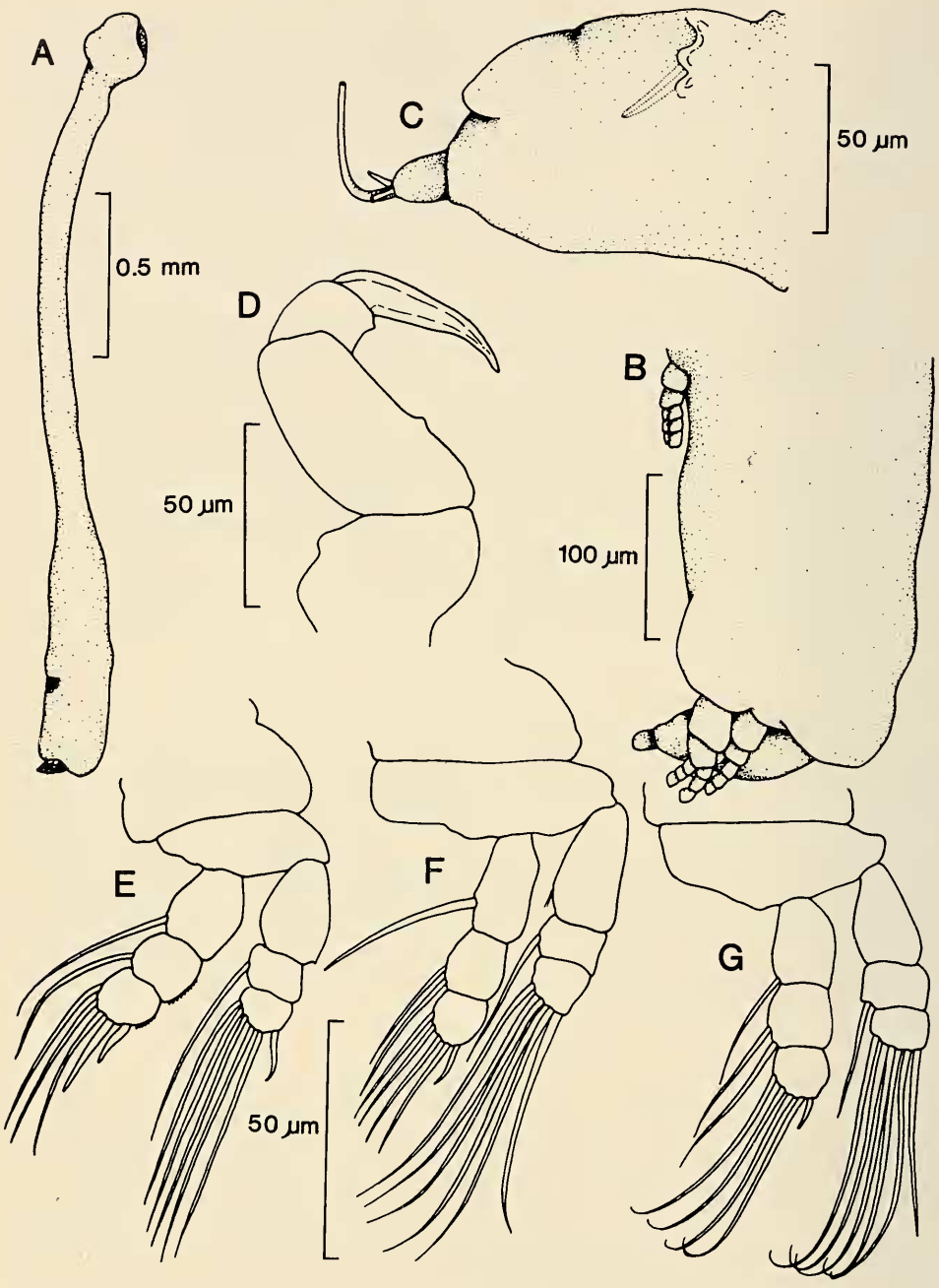

Fig. 1. Mugilicola australiensis, holotype female. **A**, Dorsal. **B**, Posterior end of trunk, lateral. **C**, Urosome, lateral. **D**, Antenna. **E**, Leg 1. **F**, Leg 2. **G**, Leg 3.

of the fused genital complex and a single abdominal segment which bears the caudal rami posteriorly. Urosome directed posteroventrally (Fig. 1**B**) and largely concealed by

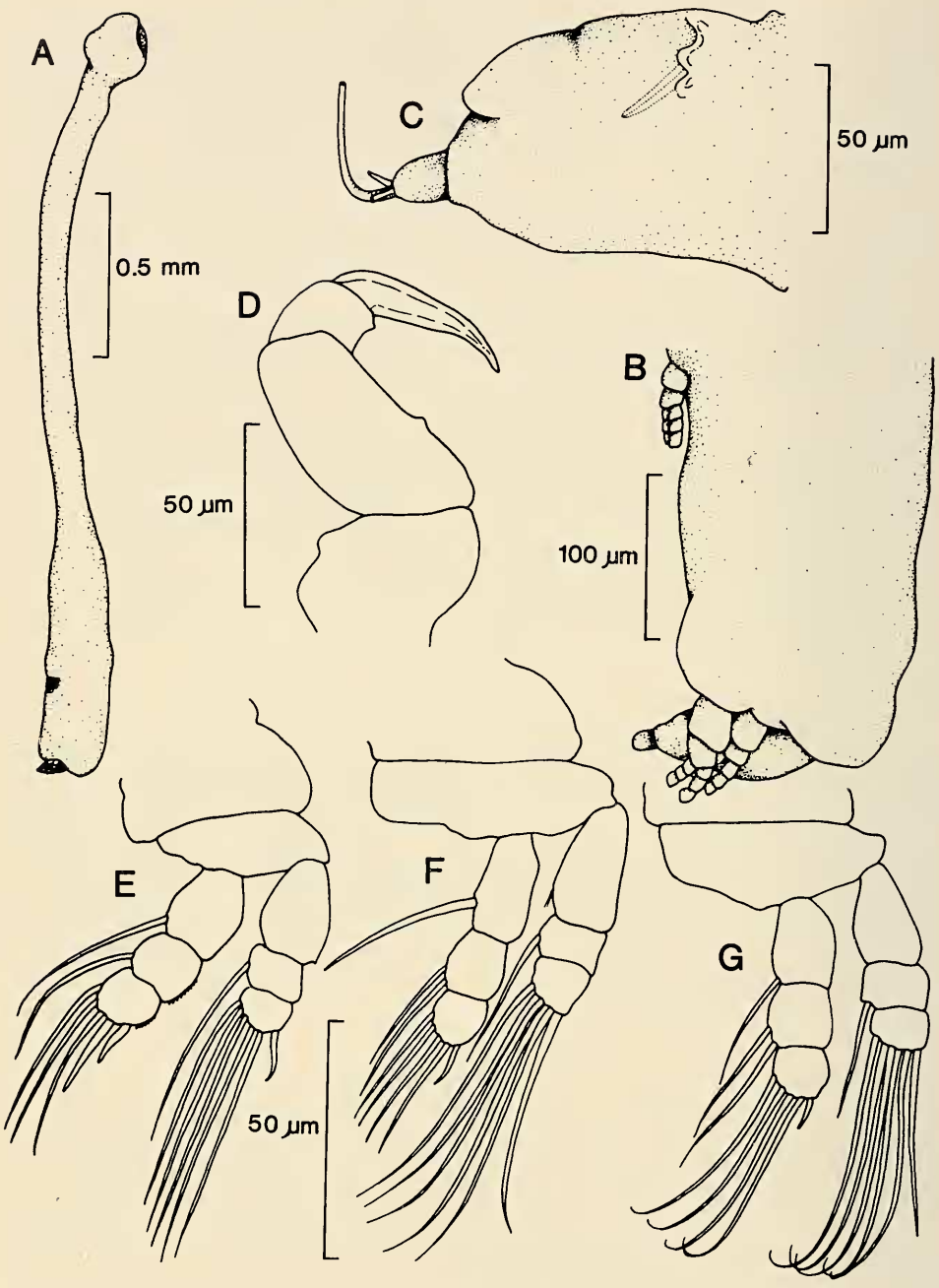

Fig. 1. Mugilicola australiensis, holotype female. **A**, Dorsal. **B**, Posterior end of trunk, lateral. **C**, Urosome, lateral. **D**, Antenna. **E**, Leg 1. **F**, Leg 2. **G**, Leg 3.

of the fused genital complex and a single abdominal segment which bears the caudal rami posteriorly. Urosome directed posteroventrally (Fig. 1**B**) and largely concealed by

posterior leg pairs, although this may be a fixation artifact. Armature of caudal rami damaged but comprising at least 3 setae. Total length of holotype ♀ 2.38 mm.

The head of the holotype is damaged and, of the cephalic appendages, only the antenna is intact. Antenna (Fig. 1**D**) subchelate, first segment unarmed, second segment bearing a small process on inner margin, third segment carrying terminal claw. Legs 1-3 biramous, with 3-segmented rami (Figs 1**E-G**); armature formula as follows:

	coxa	basis	endopod	exopod
leg 1	0-0	0-0	0-1;0-1;0,II,3	0-0;0-1;0,I,5
leg 2	0-0	0-0	0-1;0-2;0,I,4	0-0;0-1;0,1,5
leg 3	0-0	0-0	0-1;0-2;0,I,4	0-0;0-1;0,1,5

Terminal armature element on third exopod segment of legs 2 and 3 setiform. Legs 4 and 5 absent. Leg 6 forming an unarmed plate serving to close each genital aperture.

Etymology: The specific name, *australiensis*, is derived from the type-locality.

Remarks: The new species can be distinguished from its congeners by its general facies. It differs from *M. smithae* Jones and Hine, 1978 in the shape of its head, *M. smithae* being provided with trilobate posterolateral processes on its head. It differs from *M. bulbosus* Tripathi, 1960 in having a relatively longer and narrower neck and in the relatively small size of its urosome. These three species also show differences in the armature of their swimming legs. *M. bulbosus* and the new species have 2 setae on the second endopod segment of leg 2 whereas *M. smithae* apparently has only one. There may, however, be an error in the labelling of the legs in Jones and Hine (1978) as it is very unusual in copepods in general for leg 2 to have only 1 seta on this segment when legs 1 and 3 have 2 setae. *M. bulbosus* has one armature element less on the apex of the endopod of all three legs than the new species. *Mugilicola* species can be distinguished from *Paeonodes* species by the number of swimming legs, 3 in the former and 4 in the latter.

Discussion

The genera *Mugilicola, Paeonodes* and *Therodamas* all possess mouthparts of the basic ergasilid type (see Tripathi, 1960; Hewitt, 1969; Cressey, 1972). The mandible is falcate with a reduced palp, the maxillule is a lobe bearing 2 setae, and the maxilla is two-segmented with the second segment armed with many small spinules. The maxilliped is absent in the adult female. The antennule is 5-segmented in all three genera. The antenna has 3 segments plus a terminal claw in *Mugilicola* and *Paeonodes* but in *Therodamas* the 3 segments are fused into a single robust basal segment bearing the terminal claw. The swimming legs are biramous in all three genera but *Mugilicola* has only 3 pairs rather than 4. Apart from the derived condition of the antenna in *Therodamas* all of these characters occur within the Ergasilidae.

Therodamas, Mugilicola and *Paeonodes* differ in tagmosis from all the genera included in the Ergasilidae by Kabata (1979) in their possession of a long neck. It was this character more than any other upon which Tripathi (1960) based the family Therodamasidae. However, the neck of *Therodamas* is not homologous with that of *Mugilicola* and *Paeonodes*. The neck of the latter two genera is postcephalic in origin whereas that of *Therodamas* is cephalic, separating the antennae from the oral region (Cressey, 1972). The common possession of a postcephalic neck is a synapomorphy between *Mugilicola* and *Paeonodes*.

The possession of typical ergasilid cephalic appendages and the lack of a maxilliped in the adult female are the diagnostic apomorphies of the family Ergasilidae. These characters are sufficient to place *Therodamas, Mugilicola* and *Paeonodes* in that family. They are highly derived mesoparasitic representatives of a family which typically

posterior leg pairs, although this may be a fixation artifact. Armature of caudal rami damaged but comprising at least 3 setae. Total length of holotype ♀ 2.38 mm.

The head of the holotype is damaged and, of the cephalic appendages, only the antenna is intact. Antenna (Fig. 1**D**) subchelate, first segment unarmed, second segment bearing a small process on inner margin, third segment carrying terminal claw. Legs 1-3 biramous, with 3-segmented rami (Figs 1**E-G**); armature formula as follows:

	coxa	basis	endopod	exopod
leg 1	0-0	0-0	0-1;0-1;0,II,3	0-0;0-1;0,I,5
leg 2	0-0	0-0	0-1;0-2;0,I,4	0-0;0-1;0,1,5
leg 3	0-0	0-0	0-1;0-2;0,I,4	0-0;0-1;0,1,5

Terminal armature element on third exopod segment of legs 2 and 3 setiform. Legs 4 and 5 absent. Leg 6 forming an unarmed plate serving to close each genital aperture.

Etymology: The specific name, *australiensis*, is derived from the type-locality.

Remarks: The new species can be distinguished from its congeners by its general facies. It differs from *M. smithae* Jones and Hine, 1978 in the shape of its head, *M. smithae* being provided with trilobate posterolateral processes on its head. It differs from *M. bulbosus* Tripathi, 1960 in having a relatively longer and narrower neck and in the relatively small size of its urosome. These three species also show differences in the armature of their swimming legs. *M. bulbosus* and the new species have 2 setae on the second endopod segment of leg 2 whereas *M. smithae* apparently has only one. There may, however, be an error in the labelling of the legs in Jones and Hine (1978) as it is very unusual in copepods in general for leg 2 to have only 1 seta on this segment when legs 1 and 3 have 2 setae. *M. bulbosus* has one armature element less on the apex of the endopod of all three legs than the new species. *Mugilicola* species can be distinguished from *Paeonodes* species by the number of swimming legs, 3 in the former and 4 in the latter.

Discussion

The genera *Mugilicola, Paeonodes* and *Therodamas* all possess mouthparts of the basic ergasilid type (see Tripathi, 1960; Hewitt, 1969; Cressey, 1972). The mandible is falcate with a reduced palp, the maxillule is a lobe bearing 2 setae, and the maxilla is two-segmented with the second segment armed with many small spinules. The maxilliped is absent in the adult female. The antennule is 5-segmented in all three genera. The antenna has 3 segments plus a terminal claw in *Mugilicola* and *Paeonodes* but in *Therodamas* the 3 segments are fused into a single robust basal segment bearing the terminal claw. The swimming legs are biramous in all three genera but *Mugilicola* has only 3 pairs rather than 4. Apart from the derived condition of the antenna in *Therodamas* all of these characters occur within the Ergasilidae.

Therodamas, Mugilicola and *Paeonodes* differ in tagmosis from all the genera included in the Ergasilidae by Kabata (1979) in their possession of a long neck. It was this character more than any other upon which Tripathi (1960) based the family Therodamasidae. However, the neck of *Therodamas* is not homologous with that of *Mugilicola* and *Paeonodes*. The neck of the latter two genera is postcephalic in origin whereas that of *Therodamas* is cephalic, separating the antennae from the oral region (Cressey, 1972). The common possession of a postcephalic neck is a synapomorphy between *Mugilicola* and *Paeonodes*.

The possession of typical ergasilid cephalic appendages and the lack of a maxilliped in the adult female are the diagnostic apomorphies of the family Ergasilidae. These characters are sufficient to place *Therodamas, Mugilicola* and *Paeonodes* in that family. They are highly derived mesoparasitic representatives of a family which typically

contains ectoparasitic forms but there is no apparent justification for their separation at the familial level.

ACKNOWLEDGEMENTS

I would like to thank Dr Klaus Rohde (University of New England) for donating this material and Drs R. J. Lincoln and Z. Kabata for their valuable comments on the manuscript.

References

CRESSEY, R. F., 1972. — *Therodamas dawsoni*, a new species of parasitic copepod (Cyclopoida: Ergasilidae) from the west coast of Panama. *Proc. Biol. Soc. Washington* 85(20): 265-270.

HEWITT, G. C., 1969. — A new species of *Paeonodes* (Therodamasidae, Cyclopoida, Copepoda) parasitic on New Zealand freshwater fish, with a re-examination of *Paeonodes exiguus* Wilson. *Zool. Publ. Vict. Univ. Wellington* 50: 32-39.

JONES, J. B., and HINE, P. M., 1978. — A new species of *Mugilicola* parasitic on South African elvers (Copepoda, Therodamasidae). *Zool. Africana* 13: 213-219.

KABATA, Z., 1979. — *Parasitic Copepoda of British Fishes.* 468 pp. London: The Ray Society.

THOMSEN, R., 1949. — Copépodos parásitos de los peces marinos del Uruguay. *Comm. Zool. Mus. Hist. Natur. Montevideo* 3(54): 1-41.

TRIPATHI, Y. R., 1960. — Parasitic copepods from Indian fishes II. Two new families Therodamasidae and Catlaphillidae. In *Libro homenaje al Dr. Eduardo Caballero y Caballero, Jubileo 1930-1960:* 543-548. Mexico: Institut Politechnico Nacional.

contains ectoparasitic forms but there is no apparent justification for their separation at the familial level.

ACKNOWLEDGEMENTS

I would like to thank Dr Klaus Rohde (University of New England) for donating this material and Drs R. J. Lincoln and Z. Kabata for their valuable comments on the manuscript.

References

CRESSEY, R. F., 1972. — *Therodamas dawsoni*, a new species of parasitic copepod (Cyclopoida: Ergasilidae) from the west coast of Panama. *Proc. Biol. Soc. Washington* 85(20): 265-270.

HEWITT, G. C., 1969. — A new species of *Paeonodes* (Therodamasidae, Cyclopoida, Copepoda) parasitic on New Zealand freshwater fish, with a re-examination of *Paeonodes exiguus* Wilson. *Zool. Publ. Vict. Univ. Wellington* 50: 32-39.

JONES, J. B., and HINE, P. M., 1978. — A new species of *Mugilicola* parasitic on South African elvers (Copepoda, Therodamasidae). *Zool. Africana* 13: 213-219.

KABATA, Z., 1979. — *Parasitic Copepoda of British Fishes.* 468 pp. London: The Ray Society.

THOMSEN, R., 1949. — Copépodos parásitos de los peces marinos del Uruguay. *Comm. Zool. Mus. Hist. Natur. Montevideo* 3(54): 1-41.

TRIPATHI, Y. R., 1960. — Parasitic copepods from Indian fishes II. Two new families Therodamasidae and Catlaphillidae. In *Libro homenaje al Dr. Eduardo Caballero y Caballero, Jubileo 1930-1960:* 543-548. Mexico: Institut Politechnico Nacional.

Seed Protein Content of Australian Species of *Acacia*

D. R. MURRAY and C. M. MCGEE

MURRAY, D. R., & MCGEE, C. M. Seed protein content of Australian species of *Acacia*. *Proc. Linn. Soc. N.S.W.* 108 (3), (1985) 1986: 187-190.

Seed samples of 26 species of *Acacia* have been milled and extracted with 5% (w/v) potassium sulphate in 0.1M sodium phosphate buffer, pH 7.0, in the presence of Polyclar AT (0.3g per g of meal). The protein concentration of the extracts was determined with a reliable assay involving the biuret reaction. The values obtained for extractable protein content ranged from 4.89% of seed weight *(Acacia victoriae)* to 14.27% *(A. alata)*.

David R. Murray and Colette M. McGee, Department of Biology, University of Wollongong, Australia 2500; *manuscript received 15 January 1985, accepted for publication 25 May 1985.*

INTRODUCTION

Acacia seeds may represent an under-utilized human food resource, especially the seeds of Australian species, as these are free of the serious neurotoxins found among the non-protein amino acids of African and Asian species (Evans *et al.*, 1977). Recently, members of the University of Sydney Human Nutrition Unit claimed that the seeds of nine species of *Acacia* eaten by Aborigines were 'strikingly nutrient rich with higher energy, protein and fat content than crops such as wheat and rice and even higher than some meats' (Anonymous, 1984).

In contrast, Murray *et al.* (1978) reported that the amount of protein extracted from seeds of *Acacia sophorae* (Labill.) R.Br. and *A. longifolia* (Andrews) Willd. accounted for only 12 to 13% of seed dry matter content. Analyses have now been performed for a total of 26 species of *Acacia*, including nine considered to be edible (e.g. see Cribb and Cribb, 1976: 78).

MATERIALS AND METHODS

Seed samples of eight edible species of *Acacia* were supplied by Mr M. L. Farrar of Nowra, N.S.W.: *A. adsurgens, A. aneura, A. cowleana, A. coriacea, A. holosericea, A. bivenosa, A. murrayana* and *A. victoriae*. Seeds of *A. tetragonophylla* were provided by Mr J. R. Maconochie of the Northern Territory Herbarium, Alice Springs. Seeds of *A. alata* were collected (by D.R.M.) in the Kooringal Forest south of Perth, W.A.

Seeds of *A. binervata, A. riceana, A. obtusifolia* and *A. triptera* were purchased from Flamingo Enterprises, Nowra, N.S.W. Seeds of *A. floribunda, A. implexa, A. lasiocarpa, A. leioderma, A. mitchellii* and *A. oxycedrus* were from Nindethana Seed Service, Narrikup, W.A. Seeds of the remaining species were from the same batches as recorded in Weder and Murray (1981) and Murray *et al.* (1978). Seed specimens from all the species studied have been retained and will be lodged with the new Wollongong Herbarium.

Extraction Procedure

Seeds (3g to 5g) were finely milled in an electric coffee grinder. Samples of meal (1.0g) were weighed and combined with 0.3g of insoluble polyvinyl pyrrolidone (Polyclar AT) as a precaution against interference by phenolic compounds from seed coats, then suspended in 10ml of 5% (w/v) K_2SO_4 in 0.1M Na phosphate, pH 7.0 (Murray, 1979). Each suspension was stirred continuously for 1h at room temperature, then squeezed through two layers of cheesecloth and centrifuged at 12,000*g* for 20 min. The volume of

Seed Protein Content of Australian Species of *Acacia*

D. R. MURRAY and C. M. MCGEE

MURRAY, D. R., & MCGEE, C. M. Seed protein content of Australian species of *Acacia*. *Proc. Linn. Soc. N.S.W.* 108 (3), (1985) 1986: 187-190.

Seed samples of 26 species of *Acacia* have been milled and extracted with 5% (w/v) potassium sulphate in 0.1M sodium phosphate buffer, pH 7.0, in the presence of Polyclar AT (0.3g per g of meal). The protein concentration of the extracts was determined with a reliable assay involving the biuret reaction. The values obtained for extractable protein content ranged from 4.89% of seed weight *(Acacia victoriae)* to 14.27% *(A. alata)*.

David R. Murray and Colette M. McGee, Department of Biology, University of Wollongong, Australia 2500; *manuscript received 15 January 1985, accepted for publication 25 May 1985.*

INTRODUCTION

Acacia seeds may represent an under-utilized human food resource, especially the seeds of Australian species, as these are free of the serious neurotoxins found among the non-protein amino acids of African and Asian species (Evans *et al.*, 1977). Recently, members of the University of Sydney Human Nutrition Unit claimed that the seeds of nine species of *Acacia* eaten by Aborigines were 'strikingly nutrient rich with higher energy, protein and fat content than crops such as wheat and rice and even higher than some meats' (Anonymous, 1984).

In contrast, Murray *et al.* (1978) reported that the amount of protein extracted from seeds of *Acacia sophorae* (Labill.) R.Br. and *A. longifolia* (Andrews) Willd. accounted for only 12 to 13% of seed dry matter content. Analyses have now been performed for a total of 26 species of *Acacia*, including nine considered to be edible (e.g. see Cribb and Cribb, 1976: 78).

MATERIALS AND METHODS

Seed samples of eight edible species of *Acacia* were supplied by Mr M. L. Farrar of Nowra, N.S.W.: *A. adsurgens, A. aneura, A. cowleana, A. coriacea, A. holosericea, A. bivenosa, A. murrayana* and *A. victoriae*. Seeds of *A. tetragonophylla* were provided by Mr J. R. Maconochie of the Northern Territory Herbarium, Alice Springs. Seeds of *A. alata* were collected (by D.R.M.) in the Kooringal Forest south of Perth, W.A.

Seeds of *A. binervata, A. riceana, A. obtusifolia* and *A. triptera* were purchased from Flamingo Enterprises, Nowra, N.S.W. Seeds of *A. floribunda, A. implexa, A. lasiocarpa, A. leioderma, A. mitchellii* and *A. oxycedrus* were from Nindethana Seed Service, Narrikup, W.A. Seeds of the remaining species were from the same batches as recorded in Weder and Murray (1981) and Murray *et al.* (1978). Seed specimens from all the species studied have been retained and will be lodged with the new Wollongong Herbarium.

Extraction Procedure

Seeds (3g to 5g) were finely milled in an electric coffee grinder. Samples of meal (1.0g) were weighed and combined with 0.3g of insoluble polyvinyl pyrrolidone (Polyclar AT) as a precaution against interference by phenolic compounds from seed coats, then suspended in 10ml of 5% (w/v) K_2SO_4 in 0.1M Na phosphate, pH 7.0 (Murray, 1979). Each suspension was stirred continuously for 1h at room temperature, then squeezed through two layers of cheesecloth and centrifuged at 12,000*g* for 20 min. The volume of

recovered supernatant was measured and three aliquots (50μl or 100μl) were removed for protein estimation.

Almost all of the extractable protein is removed with a single extraction step (Murray *et al.*, 1978: 763), but for the samples of edible species supplied by Mr M. L. Farrar, a second extraction was performed by grinding the squeezed material in a mortar with a further 10ml of extraction medium. This homogenate was squeezed through cheesecloth and centrifuged as before. The two supernatants were pooled, mixed thoroughly, and sampled for protein determination as above.

Protein Determination

The aliquots removed for protein determination were treated with ethanol (final concentration 80%, v/v). The resulting precipitates were collected by centrifugation and washed once by resuspension to remove soluble amino nitrogen. The precipitates were collected again by centrifugation, redissolved in 0.25M NaOH, and treated with biuret reagent (Gornall *et al.*, 1949). The absorbance of each tube was read at 540nm. Bovine serum albumin, treated to remove lipids, was used as a reference standard (Collier and Murray, 1977; Murray *et al.*, 1978; Murray, 1979).

Results and Discussion

The extractable protein contents of the seeds of 26 species of *Acacia* are presented in Table 1. As a proportion of seed weight, the protein content ranged from as little as 4.89% *(A. victoriae)* to as much as 14.27% *(A. alata)*. Values for protein content around 10% are common, but it is not possible to conclude that high, medium or low seed protein contents are related to the systematic treatment of an individual species. Of the species considered to be edible, *A. sophorae* (Cribb and Cribb, 1976) had the highest seed protein content (Table 1, and compare Table 5 of Murray *et al.*, 1978).

The biuret method of estimating protein content has been chosen deliberately. The biuret reaction depends upon a property shared by all polypeptide chains — the possession of adjacent pairs of peptide bonds. The procedure is admirably suited to mixtures of different proteins, as a single standard protein is sufficient, and the outcome is independent of differences in amino acid composition between individual proteins.

In contrast, the Kjeldahl method for determining total nitrogen content is far too often applied uncritically to studies of plant proteins, a situation that has not altered since the beginning of this century (Petrie, 1908). In the case of bean seeds *(Phaseolus vulgaris)* a Kjeldahl nitrogen value in mg multiplied by 6.25 yields an apparent protein content that is an overestimate by a factor of two (Adriaanse *et al.*, 1969). An even greater disparity has been observed for seeds of *Cuscuta reflexa*, where a protein estimation based on Kjeldahl nitrogen determination exceeds the seeds' content of extractable protein by 5.7-fold (Rahman and Krishnan, 1971).

Petrie (1908) was probably the first person to study the nitrogenous constituents of *Acacia* seeds, and he determined that as much as 45% of the total nitrogen of mature *A. pycnantha* seeds occurs as non-protein nitrogen. Total reliance on the Kjeldahl procedure has thus led to overestimation of *Acacia* seed protein content by the group referred to previously (Anonymous, 1984). Their estimate of 24% protein for immature *A. cowleana* seeds must be compared with a value of 10.42% for mature seeds of this species (Table 1). The range of 17% to 27% protein content for seeds of all the species in this group's sample is clearly extravagant. We question their view that 'many bushfoods appear to be richer sources of nutrients than similar cultivated plants' (Anonymous, 1984). The quantity, the quality and the availability of proteins from seeds are all factors that need to be assessed. At this stage little is known about any of these properties for the seed

recovered supernatant was measured and three aliquots (50μl or 100μl) were removed for protein estimation.

Almost all of the extractable protein is removed with a single extraction step (Murray *et al.*, 1978: 763), but for the samples of edible species supplied by Mr M. L. Farrar, a second extraction was performed by grinding the squeezed material in a mortar with a further 10ml of extraction medium. This homogenate was squeezed through cheesecloth and centrifuged as before. The two supernatants were pooled, mixed thoroughly, and sampled for protein determination as above.

Protein Determination

The aliquots removed for protein determination were treated with ethanol (final concentration 80%, v/v). The resulting precipitates were collected by centrifugation and washed once by resuspension to remove soluble amino nitrogen. The precipitates were collected again by centrifugation, redissolved in 0.25M NaOH, and treated with biuret reagent (Gornall *et al.*, 1949). The absorbance of each tube was read at 540nm. Bovine serum albumin, treated to remove lipids, was used as a reference standard (Collier and Murray, 1977; Murray *et al.*, 1978; Murray, 1979).

Results and Discussion

The extractable protein contents of the seeds of 26 species of *Acacia* are presented in Table 1. As a proportion of seed weight, the protein content ranged from as little as 4.89% *(A. victoriae)* to as much as 14.27% *(A. alata)*. Values for protein content around 10% are common, but it is not possible to conclude that high, medium or low seed protein contents are related to the systematic treatment of an individual species. Of the species considered to be edible, *A. sophorae* (Cribb and Cribb, 1976) had the highest seed protein content (Table 1, and compare Table 5 of Murray *et al.*, 1978).

The biuret method of estimating protein content has been chosen deliberately. The biuret reaction depends upon a property shared by all polypeptide chains — the possession of adjacent pairs of peptide bonds. The procedure is admirably suited to mixtures of different proteins, as a single standard protein is sufficient, and the outcome is independent of differences in amino acid composition between individual proteins.

In contrast, the Kjeldahl method for determining total nitrogen content is far too often applied uncritically to studies of plant proteins, a situation that has not altered since the beginning of this century (Petrie, 1908). In the case of bean seeds *(Phaseolus vulgaris)* a Kjeldahl nitrogen value in mg multiplied by 6.25 yields an apparent protein content that is an overestimate by a factor of two (Adriaanse *et al.*, 1969). An even greater disparity has been observed for seeds of *Cuscuta reflexa*, where a protein estimation based on Kjeldahl nitrogen determination exceeds the seeds' content of extractable protein by 5.7-fold (Rahman and Krishnan, 1971).

Petrie (1908) was probably the first person to study the nitrogenous constituents of *Acacia* seeds, and he determined that as much as 45% of the total nitrogen of mature *A. pycnantha* seeds occurs as non-protein nitrogen. Total reliance on the Kjeldahl procedure has thus led to overestimation of *Acacia* seed protein content by the group referred to previously (Anonymous, 1984). Their estimate of 24% protein for immature *A. cowleana* seeds must be compared with a value of 10.42% for mature seeds of this species (Table 1). The range of 17% to 27% protein content for seeds of all the species in this group's sample is clearly extravagant. We question their view that 'many bushfoods appear to be richer sources of nutrients than similar cultivated plants' (Anonymous, 1984). The quantity, the quality and the availability of proteins from seeds are all factors that need to be assessed. At this stage little is known about any of these properties for the seed

TABLE 1

Extractable Protein Content of Acacia *Seeds*

Species	Mean seed mass (mg)	Protein content: mg per seed	% of seed weight
Botryocephalae			
A. decurrens Willd.	13.1	1.35	10.34
A. elata A. Cunn. ex Benth.	43.2	3.69	8.55
Uninerves: Racemosae			
A. bivenosa DC[1,2]	34.9	2.53	7.24
A. murrayana F. Muell. ex Benth.[1]	29.4	2.01	6.84
A. victoriae Benth.[1]	26.6	1.30	4.89
Phyllodinous Species of Indistinct Alliance			
A. binervata DC[3]	20.6	1.72	8.35
A. tetragonophylla F. Muell.	30.7	4.06	13.23
Plurinerves			
A. coriacea DC[1]	70.1	4.82	6.88
A. implexa Benth.	20.8	1.80	8.67
A. melanoxylon R.Br. ex Ait.	15.2	1.21	7.94
Juliflorae (with Spicatae)			
A. adsurgens Maiden & Blakely[1]	7.9	0.68	8.61
A. floribunda (Vent.) Willd.	7.1	0.69	9.72
A. longifolia (Andr.) Willd.[4]	24.6	3.20	13.02
A. sophorae (Labill.) R.Br.[1,5]	37.7	4.70	12.47
A. obtusifolia A. Cunn.	14.2	1.63	11.50
A. oxycedrus Sieb. ex DC	19.6	1.77	9.01
A. riceana Henslow	9.9	0.99	10.03
A. triptera Benth.	11.1	1.21	10.90
A. aneura F. Muell. ex Benth.[1]	18.2	1.97	10.82
A. cowleana Tate[1]	11.5	1.20	10.42
A. holosericea A. Cunn. ex G. Don.[1]	11.4	1.06	9.32
Pulchellae and *A. alata*			
A. alata R.Br.[6]	10.0	1.43	14.27
A. drummondii Lindl.	2.66	0.31	11.66
A. lasiocarpa Benth.	4.52	0.44	9.81
A. leioderma Maslin	5.26	0.61	11.61
Other Bipinnatae			
A. mitchellii Benth.[6]	14.3	1.49	10.41

1 considered edible.
2 = *A. ligulata*, see Pedley (1979).
3 see Tindale and Roux (1969, 1974).
4 freshly milled sample from batch L1(2), Murray *et al.* (1978).
5 freshly milled sample from batch S3, Murray *et al.* (1978).
6 for discussion, see Guinet *et al.* (1980), Murray and Weder (1983).

proteins of native legumes. It may be assumed that the contents of essential sulphur-containing amino acids in *Acacia* seed globulins are very low, as values for these amino acids were not included in the analyses reported by Pettigrew and Watson (1975). The best-balanced protein sources from seeds are to be found among the albumin fractions of some of the cultivated legumes (for review, see Murray, 1984a,b).

Detailed electrophoretic studies on the seed proteins of the *Acacia* species listed in this paper will be the subject of a future communication.

TABLE 1

Extractable Protein Content of Acacia *Seeds*

Species	Mean seed mass (mg)	Protein content: mg per seed	% of seed weight
Botryocephalae			
A. decurrens Willd.	13.1	1.35	10.34
A. elata A. Cunn. ex Benth.	43.2	3.69	8.55
Uninerves: Racemosae			
A. bivenosa DC[1,2]	34.9	2.53	7.24
A. murrayana F. Muell. ex Benth.[1]	29.4	2.01	6.84
A. victoriae Benth.[1]	26.6	1.30	4.89
Phyllodinous Species of Indistinct Alliance			
A. binervata DC[3]	20.6	1.72	8.35
A. tetragonophylla F. Muell.	30.7	4.06	13.23
Plurinerves			
A. coriacea DC[1]	70.1	4.82	6.88
A. implexa Benth.	20.8	1.80	8.67
A. melanoxylon R.Br. ex Ait.	15.2	1.21	7.94
Juliflorae (with Spicatae)			
A. adsurgens Maiden & Blakely[1]	7.9	0.68	8.61
A. floribunda (Vent.) Willd.	7.1	0.69	9.72
A. longifolia (Andr.) Willd.[4]	24.6	3.20	13.02
A. sophorae (Labill.) R.Br.[1,5]	37.7	4.70	12.47
A. obtusifolia A. Cunn.	14.2	1.63	11.50
A. oxycedrus Sieb. ex DC	19.6	1.77	9.01
A. riceana Henslow	9.9	0.99	10.03
A. triptera Benth.	11.1	1.21	10.90
A. aneura F. Muell. ex Benth.[1]	18.2	1.97	10.82
A. cowleana Tate[1]	11.5	1.20	10.42
A. holosericea A. Cunn. ex G. Don.[1]	11.4	1.06	9.32
Pulchellae and *A. alata*			
A. alata R.Br.[6]	10.0	1.43	14.27
A. drummondii Lindl.	2.66	0.31	11.66
A. lasiocarpa Benth.	4.52	0.44	9.81
A. leioderma Maslin	5.26	0.61	11.61
Other Bipinnatae			
A. mitchellii Benth.[6]	14.3	1.49	10.41

1 considered edible.
2 = *A. ligulata*, see Pedley (1979).
3 see Tindale and Roux (1969, 1974).
4 freshly milled sample from batch L1(2), Murray *et al.* (1978).
5 freshly milled sample from batch S3, Murray *et al.* (1978).
6 for discussion, see Guinet *et al.* (1980), Murray and Weder (1983).

proteins of native legumes. It may be assumed that the contents of essential sulphur-containing amino acids in *Acacia* seed globulins are very low, as values for these amino acids were not included in the analyses reported by Pettigrew and Watson (1975). The best-balanced protein sources from seeds are to be found among the albumin fractions of some of the cultivated legumes (for review, see Murray, 1984a,b).

Detailed electrophoretic studies on the seed proteins of the *Acacia* species listed in this paper will be the subject of a future communication.

ACKNOWLEDGEMENTS

We thank all those who supplied seeds, the Joyce W. Vickery Scientific Research Fund (Linnean Society of N.S.W.) and the Australian Biological Resources Study for financial support. Thanks are also due to F. Vairinhos, Miss J. Plummer and Mrs M. Cordova-Edwards for assistance (1981, 1982) and Prof. J. S. Pate for hospitality during a visit to Perth by D.R.M. in 1982.

References

ADRIAANSE, A., KLOP, W., and ROBBERS, J. E., 1969. — Characterization of *Phaseolus vulgaris* cultivars by their electrophoretic patterns. *J. Sci. Food Agric.* 20: 647-650.

ANONYMOUS, 1984. — Aboriginal bushfoods study arouses wide interest. *The Gazette and Letter to Graduates*, The University of Sydney, 4(9): 3.

COLLIER, M. D., and MURRAY, D. R., 1977. — Leucyl β-naphthylamidase activities in developing seeds and seedlings of *Pisum sativum* L. *Aust. J. Plant Physiol.* 4: 571-582.

CRIBB, A. B., and CRIBB, J. W., 1976. — *Wild Food in Australia.* Fontana edition. Sydney: W. Collins.

EVANS, C. S., QURESHI, M. Y., and BELL, E. A., 1977. — Free amino acids in the seeds of *Acacia* species. *Phytochemistry* 16: 565-570.

GORNALL, A. G., BARDAWILL, C. J., and DAVID, M. M., 1949. — Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177: 751-766.

GUINET, Ph., VASSAL, J., EVANS, C. S., and MASLIN, B. R., 1980. — *Acacia* (Mimosoideae): composition and affinities of the series Pulchellae Bentham. *Bot. J. Linn. Soc. Lond.* 80: 53-68.

MURRAY, D. R., 1979. — The seed proteins of kowhai, *Sophora microphylla* Ait. *Z. Pflanzenphysiol.* 93: 423-428.

——, 1984a. — The Seed and Survival. *In* MURRAY, D. R., (ed.), *Seed Physiology.* Volume 1. *Development*: 1-40. Sydney: Academic Press.

——, 1984b. — Accumulation of Seed Reserves of Nitrogen. *In* MURRAY, D. R., (ed.), *Seed Physiology.* Volume 1. *Development:* 83-137. Sydney: Academic Press.

——, and WEDER, J. K. P., 1983. — Seed proteinase inhibitors of Pulchellae, *Acacia mitchellii* Benth. and *A. alata* R.Br.: exclusion of *A. mitchellii* from Pulchellae. *Aust. J. Bot.* 31: 119-124.

——, ASHCROFT, W. J., SEPPELT, R. D., and LENNOX, F. G., 1978. — Comparative biochemical and morphological studies of *Acacia sophorae* (Labill.) R.Br. and *A. longifolia* (Andrews) Willd. *Aust. J. Bot.* 26: 755-771.

PEDLEY, L., 1979. — A revision of *Acacia* Mill. in Queensland. Part 2. *Austrobaileya* 1(3): 237-337.

PETRIE, J. M., 1908. — The role of nitrogen and its compounds in plant-metabolism. Part ii. The non-protein nitrogen in seeds. *Proc. Linn. Soc. N.S.W.* 33: 835-844.

PETTIGREW, C. J., and WATSON, L., 1975. — Taxonomic patterns in amino acids of *Acacia* seed globulins. *Phytochemistry* 14: 2623-2625.

RAHMAN, Q., and KRISHNAN, P. S., 1971. — Phosphorus, nitrogen and carbohydrate composition of the seeds of *Cuscuta. Phytochemistry* 10: 1751-1757.

TINDALE, M. D., and ROUX, D. G., 1969. — A phytochemical survey of the Australian species of *Acacia. Phytochemistry* 8: 1113-1127.

——, and ——, 1974. — An extended phytochemical survey of Australian species of *Acacia*: chemotaxonomic and phylogenetic aspects. *Phytochemistry* 13: 829-839.

WEDER, J. K. P., and MURRAY, D. R., 1981. — Distribution of proteinase inhibitors in seeds of Australian *Acacias. Z. Pflanzenphysiol.* 103: 317-322.

ACKNOWLEDGEMENTS

We thank all those who supplied seeds, the Joyce W. Vickery Scientific Research Fund (Linnean Society of N.S.W.) and the Australian Biological Resources Study for financial support. Thanks are also due to F. Vairinhos, Miss J. Plummer and Mrs M. Cordova-Edwards for assistance (1981, 1982) and Prof. J. S. Pate for hospitality during a visit to Perth by D.R.M. in 1982.

References

ADRIAANSE, A., KLOP, W., and ROBBERS, J. E., 1969. — Characterization of *Phaseolus vulgaris* cultivars by their electrophoretic patterns. *J. Sci. Food Agric.* 20: 647-650.

ANONYMOUS, 1984. — Aboriginal bushfoods study arouses wide interest. *The Gazette and Letter to Graduates*, The University of Sydney, 4(9): 3.

COLLIER, M. D., and MURRAY, D. R., 1977. — Leucyl β-naphthylamidase activities in developing seeds and seedlings of *Pisum sativum* L. *Aust. J. Plant Physiol.* 4: 571-582.

CRIBB, A. B., and CRIBB, J. W., 1976. — *Wild Food in Australia.* Fontana edition. Sydney: W. Collins.

EVANS, C. S., QURESHI, M. Y., and BELL, E. A., 1977. — Free amino acids in the seeds of *Acacia* species. *Phytochemistry* 16: 565-570.

GORNALL, A. G., BARDAWILL, C. J., and DAVID, M. M., 1949. — Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177: 751-766.

GUINET, Ph., VASSAL, J., EVANS, C. S., and MASLIN, B. R., 1980. — *Acacia* (Mimosoideae): composition and affinities of the series Pulchellae Bentham. *Bot. J. Linn. Soc. Lond.* 80: 53-68.

MURRAY, D. R., 1979. — The seed proteins of kowhai, *Sophora microphylla* Ait. *Z. Pflanzenphysiol.* 93: 423-428.

——, 1984a. — The Seed and Survival. *In* MURRAY, D. R., (ed.), *Seed Physiology.* Volume 1. *Development*: 1-40. Sydney: Academic Press.

——, 1984b. — Accumulation of Seed Reserves of Nitrogen. *In* MURRAY, D. R., (ed.), *Seed Physiology.* Volume 1. *Development:* 83-137. Sydney: Academic Press.

——, and WEDER, J. K. P., 1983. — Seed proteinase inhibitors of Pulchellae, *Acacia mitchellii* Benth. and *A. alata* R.Br.: exclusion of *A. mitchellii* from Pulchellae. *Aust. J. Bot.* 31: 119-124.

——, ASHCROFT, W. J., SEPPELT, R. D., and LENNOX, F. G., 1978. — Comparative biochemical and morphological studies of *Acacia sophorae* (Labill.) R.Br. and *A. longifolia* (Andrews) Willd. *Aust. J. Bot.* 26: 755-771.

PEDLEY, L., 1979. — A revision of *Acacia* Mill. in Queensland. Part 2. *Austrobaileya* 1(3): 237-337.

PETRIE, J. M., 1908. — The role of nitrogen and its compounds in plant-metabolism. Part ii. The non-protein nitrogen in seeds. *Proc. Linn. Soc. N.S.W.* 33: 835-844.

PETTIGREW, C. J., and WATSON, L., 1975. — Taxonomic patterns in amino acids of *Acacia* seed globulins. *Phytochemistry* 14: 2623-2625.

RAHMAN, Q., and KRISHNAN, P. S., 1971. — Phosphorus, nitrogen and carbohydrate composition of the seeds of *Cuscuta. Phytochemistry* 10: 1751-1757.

TINDALE, M. D., and ROUX, D. G., 1969. — A phytochemical survey of the Australian species of *Acacia. Phytochemistry* 8: 1113-1127.

——, and ——, 1974. — An extended phytochemical survey of Australian species of *Acacia*: chemotaxonomic and phylogenetic aspects. *Phytochemistry* 13: 829-839.

WEDER, J. K. P., and MURRAY, D. R., 1981. — Distribution of proteinase inhibitors in seeds of Australian *Acacias. Z. Pflanzenphysiol.* 103: 317-322.